Environment and Natural Resources Trust Fund
2014 Request for Proposals (RFP)

Project Title: **ENRTF ID:** **048-B**

Estrogen exposure analyses in Minnesotas Shallow Lake Wildlife

Category: B. Water Resources

Total Project Budget: $ 136000

Proposed Project Time Period for the Funding Requested: 3 Years, July 2014 - June 2017

Summary:

Using biological samples already gathered from shallow lakes across Minnesota, we will determine whether environmental estrogen exposure impacts aquatic wildlife, and make recommendations about land and lake management.

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Sponsoring Organization: St. Thomas University

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Web Address

Location

Region: Statewide

County Name: Statewide

City / Township:

_____ Funding Priorities	_____ Multiple Benefits	_____ Outcomes	_____ Knowledge Base
_____ Extent of Impact	_____ Innovation	_____ Scientific/Tech Basis	_____ Urgency
_____ Capacity Readiness	_____ Leverage	_____ Employment	_____ TOTAL _____%

PROJECT TITLE: Estrogen exposure analyses in Minnesota's shallow lake wildlife

I. PROJECT STATEMENT

Minnesota's smaller lakes play an important role in the ecosystem by providing clean water, recharging groundwater stores, and sequestering chemical and soil runoff. These lakes also benefit citizens, both by providing opportunities for recreation (e.g., fishing, swimming) and by providing economic value as a site for various commercial ventures (e.g., summer camps, commercial fisheries). Endocrine-disrupting contaminants, including environmental estrogens (EEs) are present in Minnesota's larger lakes and streams at concentrations which have adverse impacts on wildlife. However, very little is known about the sources and effects of EEs in small, shallow lakes. Importantly, the use of surrounding land and associated lake management practices may exacerbate the effects of contaminants in these systems. Our preliminary data strongly suggest that EEs are present in Minnesota's shallow lakes, and that wildlife exposed to these contaminants exhibit changes in the nervous system that may impact survival and reproduction (see Figure 1).

The goals of this project are to: 1) Determine whether EE exposure is common for aquatic wildlife in shallow lakes; 2) Determine land-use practices that correlate with EE exposure (e.g., urban, agriculture- and forest-dominated ecosystems), and 3) identify the effects of EE exposure on the nervous system of aquatic species. These analyses will allow us to identify which land-use and shallow lake management practices are most beneficial to minimizing EE exposure, and associate EE exposure with impacts on wildlife. **The outcomes of this project directly address three 2014 LCCMR funding priorities:** 1) to protect or restore water quality by…improving water and land use practices; 2) to evaluate and identify the causes of observed changes in the health of fish and wildlife that may pertain to contaminants of emerging concern; 3) to protect the health of humans and aquatic and terrestrial species by advancing the development of standards for contaminants.

We will achieve these goals by 1) measuring blood vitellogenin levels (a quantifiable indicator of EE exposure) in turtles from approximately 50 shallow lakes across five geographic regions of MN (see map); 2) testing whether exposure to EEs is related to land use by combining vitellogenin data with watershed data previously obtained; 3) analyzing brain structures associated with foraging and reproductive behavior in the brains of turtles for which blood vitellogenin levels are available.

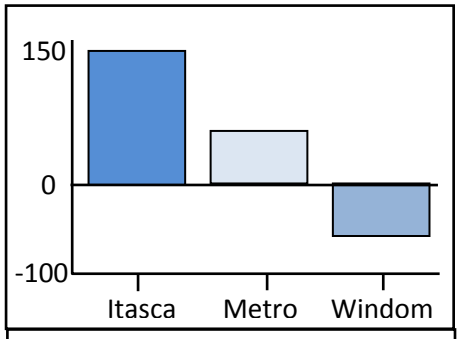


Fig. 1 Preliminary data showing the difference in the size of the NPV in male and female turtle brains. The structure usually is larger in males, but this sex difference is smaller in turtles from lakes in Metro areas compared to Itasca County, and is inverted in turtles from lakes in Cottonwood County (Windom). Size differences are expressed in thousands of square microns (area).

The proposed work is an important contribution to our understanding of the effects of contamination in Minnesota's shallow lakes by estrogen and estrogen-activating compounds. Moreover, the proposed project leverages over $100,000 of funding and in-kind services, in addition to work already performed by faculty and students at the University of St. Thomas (including sample collection, preliminary specimen preparation and analyses). Further, this study takes advantage of recently-obtained, up-to-date GIS data on land use and water quality. Thus, the proposal provides a high impact for relatively low cost, to deliver an important investigation of how land- and lake-management practices correlate with exposure and effects of EEs.

II. DESCRIPTION OF PROJECT ACTIVITIES

Activity 1: Assess the distribution of EE exposure levels in shallow lake wildlife **Budget: $60,000**

To assess EE exposure, we will develop an assay to examine vitellogenin levels in painted turtles (*Chrysemys picta*), which are long-lived, widely distributed, unlikely to be impacted by low oxygen levels, and hold value to both nature lovers and commercial fisheries. Samples of turtle blood have already been collected from shallow lakes representing distinct watersheds and land use patterns (e.g., wetlands, forest, agricultural, urban, etc.).

Outcome	Completion Date
1. Develop an assay to examine vitellogenin levels in painted turtles (*Chrysemys picta*)	April, 2015
2. Correlate vitellogenin levels with lakes impacted by environmental estrogens	February, 2016
3. Disseminate methods and data related to painted turtle vitellogenin assay (ongoing)	June, 2017

Activity 2: Identify use and management practices that reduce EE exposure **Budget: $28,000**
We will integrate EE exposure data collected in Activity 1 with a previously-collected GIS data set containing information about ecological and land-management to test whether watershed land use is related to EE levels in turtles, and assess whether these relationships vary across the state. This will help us facilitate identification of practices beneficial for reducing EE contamination

Outcome	Completion Date
1. Identify land- and water-management practices associated with EE exposure	April, 2016
2. Draft recommendations to share with managers (DNR, MPCA) and citizens (ongoing)	June, 2017

Activity 3: Determine the effects of EE exposure on nervous system structures **Budget: $48,000**
We will analyze brain regions associated with foraging and reproductive behavior in turtles for which blood vitellogenin levels are available from Activity 1. This will include examining the *nucleus paraventricularis* (NPV), a structure that is sexually dimorphic and may be related to reproductive behavior in the turtle. Preliminary results (Fig. 1) show that the difference in size of this structure between males and females varies widely throughout the state. These results, taken together with EE exposure data, will suggest whether exposure to EEs might affect behavior related to survival and reproduction in this species.

Outcome	Completion Date
1. Identify brain structures impacted by EE exposure	August, 2016
2. Disseminate findings to managers, citizens and scientists about the impact of EE exposure to facilitate development of exposure standards (ongoing)	June, 2017

III. PROJECT STRATEGY

A. Project Team/Partners

The project team will be led by Dr. Kurt R. Illig, Assistant Professor of Biology and Director of Neuroscience at the University of St. Thomas. He will be in charge of data collection and analysis, and will direct a team of undergraduate students who will assist in these efforts. In addition, Dr. Illig and the Biology Department will contribute over $100,000 worth of equipment use, supplies and labor in support of this project. Dr. Timothy Lewis and Dr. Kyle Zimmer at the University of St. Thomas will help merge results with existing GIS data. Dr. Stephen E. Bartell of Normandale College, who has developed vitellogenin assays for multiple non-model aquatic species in Minnesota, will be contracted to develop such an assay for the painted turtle, and will be paid for his work.

B. Timeline Requirements

To ensure timeliness and compatibility with existing GIS data on land management and watershed use, the project should be carried out as soon as possible. Preparation and analysis of brain tissue is the most time-consuming aspect of the proposal and is already taking place in Dr. Illig's laboratory, and will continue until the end of the project. The development and implementation of the vitellogenin assay will begin immediately upon receipt of funding, and results of these assays will be used to inform analyses and results of neural data. We expect that the project as proposed will be completed within three years.

C. Long-Term Strategy and Future Funding Needs

Although the proposed project is not likely to require more than three years, we expect that interesting results will lead to many further questions. For example, if preliminary results hold and it is found that EE exposure leads to differences in brain structures related to feeding and reproduction (see Fig. 1), follow-up studies will be required to examine questions such as: 1) What levels of EE exposure cause behavioral effects? 2) Are such effects seen in other aquatic species? 3) Does EE exposure threaten the long-term health of aquatic organisms that are important for the health of Minnesota's lakes and economy? We also may wish to expand the study by collecting organisms from more lakes. Future projects addressing such questions will likely be of interest to LCCMR and to funding agencies outside of Minnesota (e.g., the US Environmental Protection Agency, National Science Foundation, the National Institutes of Health); funding for such projects will be sought from these sources.

2014 Detailed Project Budget

Project Title: Estrogen exposure analyses in Minnesota's shallow lake wildlife

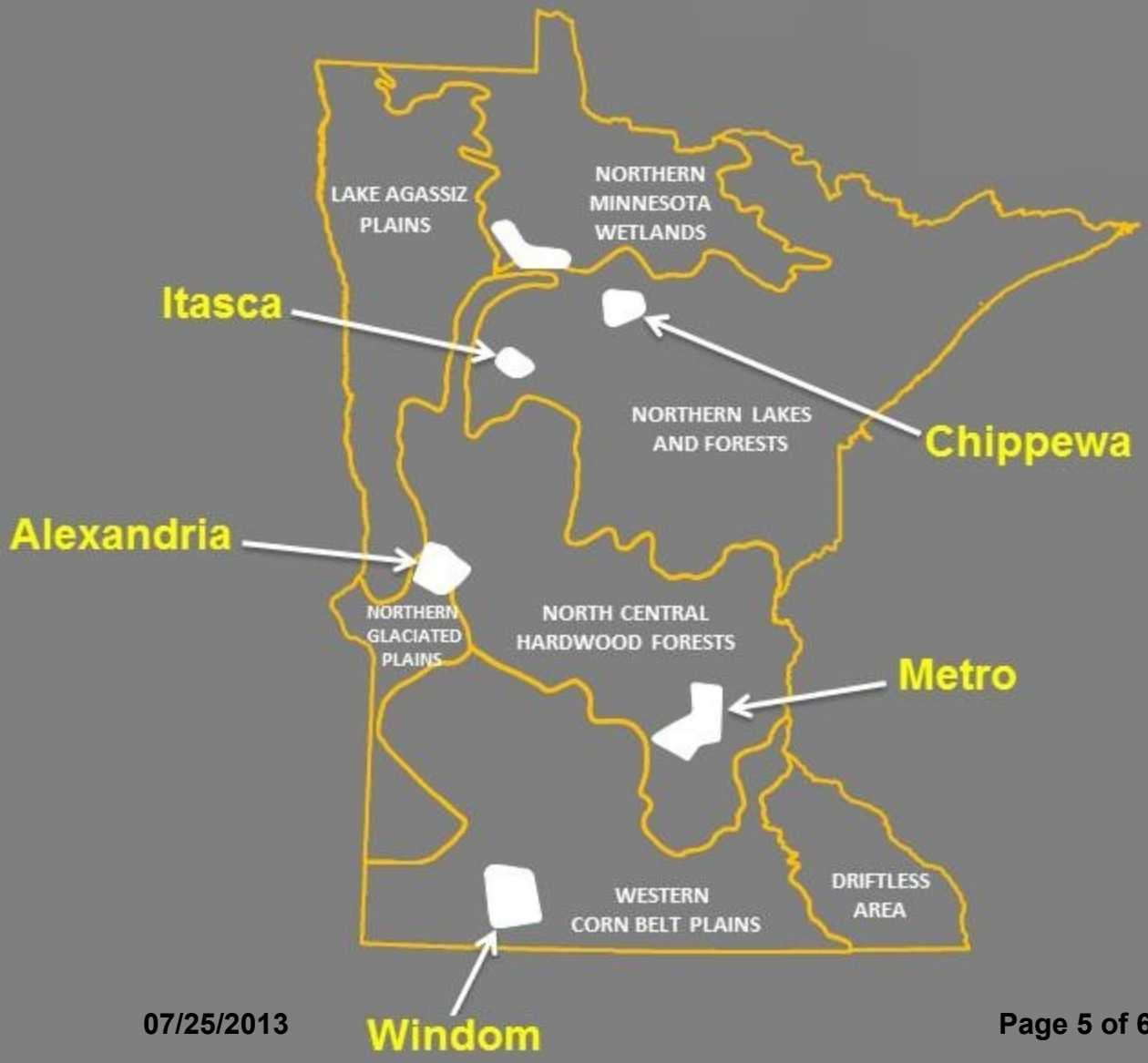
IV. TOTAL ENRTF REQUEST BUDGET 3 years

BUDGET ITEM	AMOUNT
Personnel: Kurt Illig, overall project director; brain morphology work; personnel training and management; data analysis, writing and dissemination; four months work over three years, 8% employment over the three year study, 99.235 % towards salary, 7.65% FICA	$ 32,000
Personnel: 8 undergraduate researchers providing 24 person-months work over three years 99.235 % towards salary, 7.65% FICA	$ 36,000
Contracts: Stephen Bartell, Normandale Community College, VTG assay development	$ 14,000
Equipment/Tools/Supplies: *VTG development supplies and consumbles*	$ 36,000
Equipment/Tools/Supplies: *Brain structure analysis supplies and consumables*	$ 18,000
TOTAL ENVIRONMENT AND NATURAL RESOURCES TRUST FUND $ REQUEST =	$ 136,000

V. OTHER FUNDS

SOURCE OF FUNDS	AMOUNT	Status
Other Non-State $ Being Applied to Project During Project Period: University of St. Thomas funding for student reserachers	$ 18,000	*Secured*
Other State $ Being Applied to Project During Project Period: *N/A*	$ -	
In-kind Services During Project Period: extensive equipment list	$ 24,000	Secured
Remaining $ from Current ENRTF Appropriation (if applicable): *N/A*	$ -	
Funding History: *UST supplied funding for faculty research time*	$ 22,000	Secured
Funding History: *UST supplied funding for undergraduate researchers*	$ 24,000	Secured
Funding History: *UST supplied funding for equipment, consumables*	$ 16,000	Secured

Turtle samples have been collected from approximately 50 lakes in five distinct watersheds:



Kurt R. Illig, PhD, has over 20 years of experience conducting research on the nervous systems of model and non-model organisms. He obtained his PhD from the University of Wisconsin-Madison, completed a postdoctoral fellowship at the University of Wisconsin Medical School, and was a faculty member at the University of Virginia for 7 years before taking his current position at the University of St. Thomas. He has received several research grants, and published peer-reviewed publications and authored book chapters on comparative neuroscience, and has presented papers and invited talks at dozens of national and international conferences. He also has nearly five years of administrative experience managing deadlines and budgets as the Director of the Neuroscience Program at the University of St. Thomas.

Dr. Illig's research involves comparative neuroanatomy and neurophysiology, especially in the context of survival mechanisms for the individual and the species. This research has involved various collaborators including over 20 undergraduate researchers. Undergraduate students, who will be performing some of the work in this proposal, are a regular and skilled part of Dr. Illig's research team, and their inclusion in this proposal extends the value of the proposal to include an educational component. Dr. Illig has worked closely with this team for two years as they gathered initial samples and preliminary data, and their extensive experience together ensures not only an educational benefit to the students but also the clear confidence that the outcomes of this proposal will be realized.

Dr. Illig's laboratory at the University of St. Thomas is ready and well-equipped to perform the analyses in this proposal. In fact, the laboratory has already been working with many of the samples collected, and have made substantial initial progress on the proposed work (including preliminary data included in the proposal). Development of the vitellogenin assay will be contracted to a recognized and established expert off-site (Dr. Stephen Bartell) who has developed vitellogenin assays for other non-model organisms in Minnesota. Within the Biology Department at the University of St. Thomas, Dr. Illig has a team of collaborators with the skills and experience to help frame the results in the context of greater ecological questions, including Dr. Kyle Zimmer, who was instrumental in gathering much of the watershed GIS data with which the results of this project will be integrated, and Dr. Timothy Lewis, a population wildlife ecologist whose work includes Painted Turtles from Minnesota's shallow lakes.

Founded in 1885, the University of St. Thomas is Minnesota's largest private college or university, and of the largest and oldest Catholic colleges or universities in the United States. The university offers bachelor's degrees in more than 90 major and 60 minor fields of study, including degrees in Biology, Neuroscience, Environmental Science and Biochemistry.